REMARKS

Reconsideration is requested.

Claims 89 and 108 have been canceled, without prejudice, to advance prosecution. The amendment makes the the Section 112, second paragraph, rejection of claims 89 and 108 moot.

Claims 88, 101, 102, 104, 105, 107 and 109-111 are pending.

The applicants acknowledge, with appreciation, the Examiner interview of September 16, 2005. The Examiner's brief summary of the issues discussed in the Interview Summary is accurate. Much of the following remarks relating to the cited art was also discussed.

Return of an initialed copy of the attached PTO 1449 Form, which lists the attached art, pursuant to MPEP § 609, is requested.

The Section 103 rejection of claims 88-89, 101-102, 104-105 and 107-111 over Todorov (Laboratory Investigation, Vol 78, No. 1, pp 73-78, January 1998) and Murphy (Clinical Oncology, 2nd Ed. Chapter 5, 1995), is traversed. Reconsideration and withdrawal of the rejection are requested in view of the following distinguishing comments.

The claimed invention would not have been obvious from a combination of the cited art.

The presently claimed invention provides a non-invasive cytology method for identification of, for example, cervical cancer. The most common non-invasive method of cervical cancer testing is a Pap smear, which relies on visual identification of abnormal cellular morphology.

In 1943, Papanicolau and Trout introduced the Pap smear test to detect precursors of cervical cancer in women. The Pap cytological screening test has probably proved to be the most successful public health measure introduced for the prevention of cancer. Mass screening programs, in which women have cervical smear tests at least once every three to five years, have proven highly effective in some countries in reducing cervical cancer mortality and morbidity rates. In British Columbia and Finland for example, organized screening has reduced mortality rates for cervical cancer by 70%. If detected early, cervical cancer is easily treated.

In spite of these achievements, more than 50% of the hundreds of thousands of women who develop cervical cancer annually will die of the disease. Seventy five per cent of those women will be in the developing world where mass screening programs using available methodologies are non-viable because of financial constraints. Even in many developed countries, the decline of the disease in the past decade has been insignificant where the impact of cytological screening has been far less than expected.

The main reasons that cytological screening fails to detect cervical cancer are (1) the large number of false negatives results (10-30%) and (2) the large intervals between tests.

The large number of false negative results produced in the non-invasive Pap smear reflects the fact that interpretation of Pap smears is one of the most difficult of morphological exercises. The results of the non-invasive Pap smear are more difficult to interpret than those of invasive fine needle aspiration and biopsies because of the complexity and variability of the mixed cell population placed on the smear and the wide range of inflammatory and reparative processes that occur in the cervix. There are also

cyclical changes in the cellular population, pregnancy induced alterations and alterations that occur in the postmenopausal period.

Because gynaecological cytology is so difficult, the training periods for cytotechnologists are long; they require an educated student and high degree of discipline and pattern recognition skills. Even after completing an adequate training program, cytotechnologists require several years of practical experience before they can make consistently accurate judgments as to whether a Pap smear result is normal or abnormal. Similarly, although pathologists may be trained to interpret histological sections, they require specialized additional training in cytopathology to possess adequate skills to organize and supervise the cytology laboratory and to make appropriate diagnoses concerning abnormal smears.

The general difficulty of obtaining conclusive, clinically relevant results from cytolomorphologic evaluation is confirmed by Murphy et al, cited by the Examiner. Specifically, Murphy provides the following concluding remarks regarding "Cytology" (see page 82 of the cited reference):

> "Cytology has a high degree of reliability when morphologic interpretation are preformed by experienced, well-trained individuals. Notheless, an effort should always be made to confirm the diagnosis by conventional biopsy prior to definitive treatment. The reliability of fine needle aspiration, when compared with open surginal biopsy, continues to be a matter of some controversy."

Murphy further teaches the that no tumor marker has demonstrated a specificity or sensitivity that is adequate for the screening detection of malignancies in the general population. Moreover, Murphy teaches that tumor markers are detected by pathological

examination of tumor tissue by immunohistochemistry. See page 94, left column, 3rd full paragraph, of Murphy.

Murphy therefore teaches away from the measurement or detection of tumor markers in cytology samples to determine the presence or absence of dysplastic or neoplastic cells, as claimed. At best, Murphy teaches the detection of tumor markers in histology samples as a means to confirm the clinical relevance of cytomorphologic evaluation.

As noted above, a major problem associated with Pap screening is an apparently unavoidable false negative rate (10-30%). A further major problem with this traditional morphology cytology test is the relatively high cost of screening. Alternative approaches to cervical screening include HPV DNA testing and typing as either a primary screen or as a supplement to Pap smears and to use instruments that can automatically screen conventionally collected Pap smears, thus reducing the need for the relatively highly paid cytotechnologists and cytopathologists.

There have been problems of sensitivity and positive predictive value using in-situ methods for HPV DNA testing. The use of PCR for HPV DNA detection produced such high rates of HPV infection in the general population that HPV DNA testing is thought to be of questionable use for clinical screening.

The second approach involves automation. A number of companies are currently developing and marketing automated screening instruments. In general such instruments use a high resolution video scanner to capture images, which are then digitalized and analyzed with a series of algorithms, and the data are then passed through an interference network through which the machine has been trained to

distinguish between normal and abnormal cellular components. It is hoped that with further software and hardware development, automated screening can be considered for primary screening, though at the moment no devices have been approved for the pre-screening or independent screening of Pap smears by the US FDA.

The fact that companies are prepared to invest so heavily in such an expensive and complex approach in attempting to overcome problems with conventional Pap smear testing illustrates the severity of the problems and the heart-felt and urgent need for a clinically reliable and preferably non-invasive test method.

As noted above, Murphy teaches the generally held view that tumor markers or proliferation markers do not generally provide useful clinical information for the screening or detection of malignancies in the general population.

The present inventors have discovered however that minichromosome maintenance proteins (MCM2 in the case of the presently claimed invention) provide an unexpectedly valuable clinical tool in that increased expression of MCM proteins occurs in the superficial or outermost layers of dysplastic or neoplastic tissue. The present inventors have also appreciated that the increased expression of MCM proteins in these outermost layers, which are often epithelial cells, makes it more likely that MCM proteins will be easily detected in exfoliated or sloughed cells in body fluids which may be sampled by non-invasive methods.

As discussed with the Examiner during the interview, the cited primary reference, Todorov, teaches away from the presently claimed invention.

Specifically, Table 1 of Todorov teaches an overall 27% false positive rate of tumor identification when MCM2 was measured in SDS solubilized tissue which was

separated on SDS polyacrylamide gel. See Figure 1 and Table 1 of Todorov and specifically "(27)" in the third column of Table 1 of Todorov where an overall 6 positives were detected in 22 total samples of breast, colon, lung, kidney, skeletal muscle and "other" tissue.

The overall 27% false positive rate of Todorov's invasive immunoblot method would have led one of ordinary skill in the art to conclude that measurement of MCM2, at least for cervical samples, would be no more sensitive or specific for clinical use than the generally accepted non-invasive Pap smear. Measurement of MCM2 in histology samples according to Todorov therefore would not have been expected to have been a reliable confirmatory test suggested by Murphy to be required.

Moreover, the immunoblot analysis of Todorov did not distinguish between expression in layers of different tissues such that one of ordinary skill in the art would not have been motivated to determine the presence or absence of dysplastic or neoplastic cells in a cytology test sample of essentially exfoliated or sloughed cells (i.e., a sputum sample, a bronchio-alveolar lavage sample, a urine sample, a breast duct fluid sample, a brushings from the alimentary tract, a cervical cytology sample, a fecal sample, or a urine sample), as claimed.

In fact, Todorov teaches that there is a 60% false positive rate in normal colon tissue which is theorized by Todorov to be due to the "significant population of proliferating cells [in normal tissues], such as colon epithelium." See page 76, left column, 4th sentence in first full paragraph. While the 60% false positive rate for normal colon tissue was based on homogenized tissue prepared for the Western blotting described by Todorov, Todorov suggests that normal colon epithelium is a significant

source of the false positive results.[1] The suggestion by Todorov that normal colon epithelium is a significant source of the 60% false positive Western blotting results further teaches away from measuring MCM2 in cytology samples according to the presently claimed invention.

There is no suggestion in Todorov that there is a clinically significant distinction between expression of MCM2 in basal and surface layers of dysplastic or neoplastic tissue which can be exploited in a non-invasive method, as presently claimed. In contrast to Todorov, the present inventors have demonstrated that MCM2 staining of histological sections of normal tissue was only seen in the lower third of colonic crypts. See last paragraph of page 60 of the specification.

The advantages of the presently claimed method are confirmed in the attached Davies et al (Colorectal Disease, 6, 103-110 (2004)) wherein the authors report that

> "The increased frequency of Mcm2 expression in the superficial third of the glands [i.e., colon] was a consistent and uniform observation throughout the areas of active disease." See page 107, right column of Davies et al.

The authors conclude that measurement of a MCM2 could be a stool based marked for non-invasive detection of active Inflammatory Bowel Disease (IBD). Specifically, the authors state that:

> "The increased expression of Mcm2 in the superficial, and therefore luminal, aspect of the gland in active IBD suggests that cells expressing Mcm2 would be exfoliated or sloughed into faeces during periods of disease activity." See page 108, right column of Davies et al.

[1] The applicants believe that while normal colonic mucosa contain a significant proportion of proliferating cells, these cells are present at the base of the glands and not at the surface.

Davies et al further report the earlier demonstration that it is possible to use immunocytochemistry for MCM2 on colonocytes retrieved from stool to distinguish patients with colorectal cancer from healthy volunteers and those with quiescent, non-neoplastic disease of the large bowel. Id. Davies et al also report the previous demonstration that antibodies against MCM proteins can improve the sensitivity and specificity of the cervical smear test. See page 104, left column of Davies et al and reference [10] (Davies et al, Lancet (2002) 359, 1917-9) as well as Example 14, pages 53-54, of the present specification.

The presently claimed invention would not have been obvious in view of the cited art.

Murphy teaches that there are no tumor markers which have been demonstrated to be specific or sensitive enough for screening detection of malignancies. Murphy further teaches that results obtained from non-invasive cytolomorphologic evaluation must be confirmed by invasive biopsies and histology studies to be clinically relevant.

Todorov provides overall clinically unacceptable false positive results from normal tissues (i.e., 27%) and suggests that normal tissues possessing a significant population of proliferating cells, such as colon tissue, will demonstrate an even greater clinically unacceptable false positive rate (i.e., 60%). Moreover, Todorov does not teach or suggest that there is a clinically significant distinction between expression of MCM2 in basal and surface layers of dysplastic or neoplastic tissue which can be exploited in a non-invasive method, as presently claimed.

Any motivation which may have existed to combine Todorov and Murphy would have taught away from the presently claimed invention.

Withdrawal of the Section 103 rejection of the claims is requested.

The Examiner is requested to contact the undersigned in the event anything further is required to place the application in condition for allowance.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____
B. J. Sadoff
Reg. No. 36,663

BJS:
901 North Glebe Road, 11th Floor
Arlington, VA 22203-1808
Telephone: (703) 816-4000
Facsimile: (703) 816-4100